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Analysis of Human Rotavirus Strains Prevailing in Bangladesh in Relation to Nationwide Floods Brought by the 1988 Monsoon

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The virologic character of human rotavirus strains prevailing in Bangladesh was investigated in relation to the devastating nationwide floods brought by the 1988 monsoon. Human rotaviruses contained in stool specimens that were collected from inpatients with infantile and adult diarrhea in two hospitals in Mymensingh over a 13-month period (January 1988 to January 1989) and in one hospital in Dhaka over a 3-month period (February to April 1988) were examined for their subgroup, VP7 serotype, and RNA electropherotype. In concurrence with the spread of the flood (from the middle of August 1988), the number of infantile and adult diarrhea patients increased greatly. At the same time, the proportion of rotavirus-positive specimens in all diarrhea cases also increased remarkably, reaching 54 and 45% in September and October, respectively. An electrophoretic analysis of viral RNA revealed 17 distinct patterns of viral RNA (14 long and 3 short electropherotypes) and a considerable number of mixed electropherotypes, suggesting the simultaneous infection of some patients with more than two rotavirus strains. It was noteworthy that electropherotypes of rotavirus strains prevailing in the community changed considerably after the spreading of the flood and that the frequency of virus specimens showing mixed electropherotypes increased significantly during the flood period. These results suggest that sudden environmental change caused by the devastating floods seriously affected the epidemiology of rotavirus infections by increasing the opportunity of transmission of the virus and by reducing the resistance of the host to infection. In both pediatric and adult patient groups, serotypes 1 and 2 were the most frequent ones detected, followed by serotype 4. Serotype 3 was detected in a single specimen of the 99 specimens whose serotypes were determined.

Group A rotavirus is known to be the most common cause of severe diarrhea that occurs every winter among infants and young children in temperate regions (19). Group A rotavirus is characterized by three major antigens, the subgroup antigen, the VP7 serotype antigen, and the VP4 serotype antigen, and its genome consists of 11 double-stranded RNA segments (19). At least two distinct subgroup antigens (subgroups I and II) and seven different VP7 serotype antigens (serotypes 1, 2, 3, 4, 8, 9, and 12) have so far been distinguished in human rotavirus (2, 7, 15, 23, 31, 36). Human rotaviruses with subgroup I specificity possess serotype 2, 8, or 12 antigen, while viruses with subgroup II specificity have serotype 1, 3, 4, or 9 antigen (2, 7, 15, 23, 36). Although the epidemiologic importance of the four major serotypes (1 through 4) has been indicated by their worldwide distribution, the importance of strains with the newly described serotypes 8, 9, and 12 remains unknown. Recently, the presence of VP4 serotypes in human rotavirus has been clearly shown by Gorziglia et al. (12) by using antisera prepared against the VP4 proteins that had been expressed by insertion of the VP4 gene into a recombinant baculovirus. However, since the immunogenicity of the VP4 serotype-specific antigen in natural infection or in immunization with human rotavirus is usually low, the serotype specificity of human rotavirus is considered to be mostly determined by the antigenic specificity of VP7.

The understanding of the epidemiologic features of individual rotavirus serotypes prevailing worldwide, especially in developing countries, is most necessary before the introduction of a human rotavirus vaccine, which is now being developed. For this purpose, a simple method for rotavirus serotyping that does not require virus neutralization in cell culture has been badly needed. Recently, we developed an enzyme-linked immunosorbent assay (ELISA) using serotype 1-, 2-, 3-, and 4-specific neutralizing monoclonal antibodies for directly serotyping rotavirus in stool specimens (32, 34). This method and a similar method developed by other investigators have been employed successfully to serotype rotavirus strains in clinical specimens in a number of epidemiologic studies (1, 4, 13, 22, 35).

Examination of electrophoretic patterns (electropherotypes) of segmented viral RNA by polyacrylamide gel electrophoresis (PAGE) is another indispensable tool in the epidemiology of rotavirus infections (8). Molecular epidemiologic studies of rotavirus based on the identification of RNA electropherotypes of virus strains circulating in a community have also been reported by many investigators (3, 6, 9, 25, 26, 28). Our preliminary report (1) described the subgroup and serotype antigen determination of rotaviruses in stool specimens collected from diarrheic patients in Bangladesh between January and June 1988. Collection of specimens was continued until January 1989. In this study, the subgroups, serotypes, and electropherotypes of rotavirus strains prevailing in Bangladesh were examined in speci-

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mens obtained from children and adults over a 13-month period. Special attention was given in this study to the effect of the devastating nationwide flood in 1988 on the electropherotypes of genomic RNA of prevalent rotavirus strains in Bangladesh.

MATERIALS AND METHODS

Virus specimens. Stool specimens were obtained in three hospitals in Bangladesh (1). Specimens of pediatric and adult patients with diarrhea were collected from the Mymensingh Medical College (MMC) and SK hospitals, respectively, in Mymensingh between January 1988 and January 1989. Specimens from pediatric patients were also obtained from the Dhaka Medical College (DMC) hospital in Dhaka between January and June 1988. Stool suspensions of about 10% were prepared in phosphate-buffered saline, clarified, and transported to the Department of Hygiene and Epidemiology, Sapporo Medical College, Sapporo, Japan. All specimens were screened for the presence of group A rotavirus by an ELISA with the monoclonal antibodies YO-156 (reacting with the group A common antigenic epitope in the inner capsid protein VP6) and YO-2C2 (reacting with the cross-reactive neutralization epitope in the outer capsid protein VP4) (35). Specimens that reacted with either of the two antibodies were designated as containing group A rotavirus.

Subgrouping and serotyping of human rotavirus. As previously described (32, 35), subgrouping and serotyping were carried out by using an ELISA with subgroup I- and II-specific monoclonal antibodies (S2-37 and YO-5, respectively) directed at VP6 and serotype 1-, 2-, 3-, and 4-specific monoclonal antibodies (KU-4, S2-2G10, YO-1E2, and ST-2G7, respectively) directed at the major outer capsid glycoprotein VP7. The color reaction of peroxidase with its substrate (*o*-phenylenediamine dihydrochloride) was measured as A_{492}. The criteria for determining serotype were as follows: a virus was assigned to a specific serotype when the optical density value for the reaction with the monoclonal antibody corresponding to that serotype exceeded 0.2 per well and in addition, when the optical density value for the reaction corresponding to that serotype was greater than twice the value corresponding to any other serotype (35).

Polyacrylamide gel electrophoresis. Extraction of viral RNA from stool specimens was described previously (21). Electropherotyping of viral RNA was carried out in 10% polyacrylamide slab gels, and silver staining was performed as previously described (21).

Virus isolation and serotype determination of isolates. Virus isolation from selected stool specimens was carried out by using primary green monkey kidney cell cultures following the procedure described previously (37). Virus was passaged at least four times in cell culture for isolation. The serotypes of the isolates were determined by a fluorescence focus reduction neutralization test with hyperimmune serum (30) as well as by the ELISA mentioned above.

Meteorological data. The weather records for Mymensingh for the year 1988 were obtained from the Department of Irrigation and Water Management, Bangladesh Agricultural University, Mymensingh, Bangladesh.

RESULTS

Epidemiological setting relevant to the collection of virus specimens used in this study. Monthly temperature and rainfall in Mymensingh and the number of pediatric diarrhea patients admitted to the MMC hospital and of adult diarrhea patients admitted to the SK hospital by month are shown in Fig. 1a and b. In Bangladesh, the temperature is usually high between April and October and relatively low between December and February. In 1988, Bangladesh suffered record-breaking heavy rainfall brought by a monsoon. Rainfall was from 60 to more than 70 cm per month in May, June, and July (Fig. 1a). In relation to the temperature and rainfall, the relative humidity rose to more than 85% between May and September (data not shown). In consequence of the heavy rainfall, floods began to spread throughout the country from the middle of August. It was noted that the spread of flooding coincided with the increase in the numbers of pediatric and adult diarrhea patients admitted to the two hospitals: the patient numbers reached peaks in September and October in the MMC and SK hospitals, respectively (Fig. 1b).

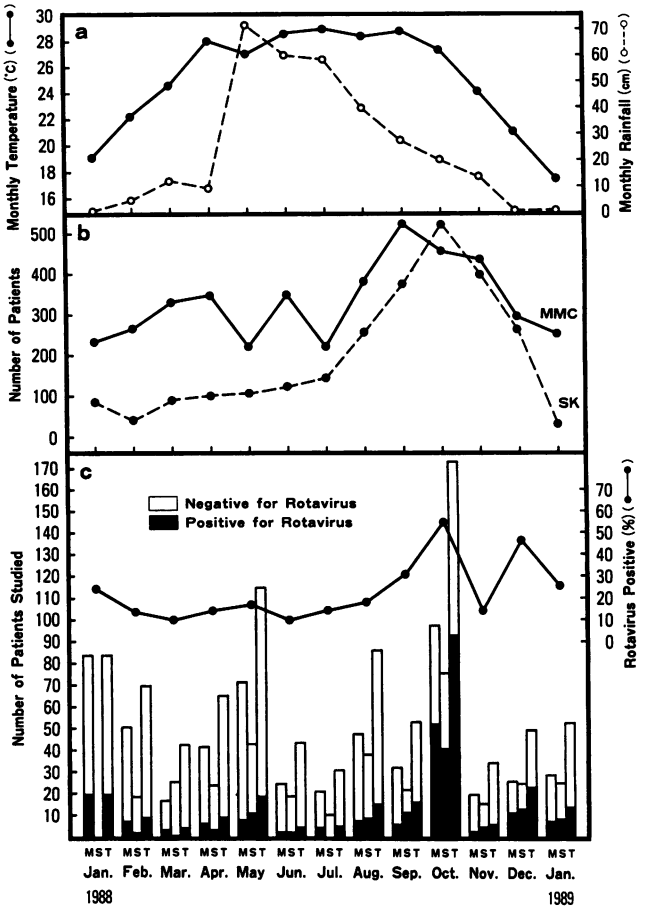

FIG. 1. Climatologic variables by month in Mymensingh (a), monthly number of diarrhea patients admitted to the two hospitals (b), and the percentage of patients with rotavirus diarrhea by month in the two hospitals in Mymensingh (c). Monthly temperature was calculated as a mean of daily average temperatures. Here, the daily average temperature was computed by averaging the temperatures at 9 o'clock a.m. and 6 o'clock p.m. every day on the basis of the weather records of Bangladesh Agricultural University. M, S, and T at the bottom in Fig. 1c indicate the number of patients in the MMC and SK hospitals and the total number of patients in the two hospitals, respectively.

From some of these patients, stool specimens were obtained and examined for the presence of rotavirus by an ELISA with group A-specific monoclonal antibodies. The

TABLE 1. Age distribution of patients with rotavirus-positive stool specimens

Hospital	No. of specimens in each age group										
	0–5 mo	6–11 mo	1 yr	2 yr	3–5 yr	6–12 yr	13–19 yr	29–29 yr	30–39 yr	40–49 yr	50+ yr
DMC	0	10	17	9	6						
MMC	3	19	23	12	23	23					
Jan.–July, 1988	2	6	9	3	2	1					
Aug. 1988–Jan. 1989	1	13	14	9	21	22					
SK							15	35	31	9	6

number of rotavirus-positive and -negative specimens obtained in the MMC and SK hospitals and the percentage of rotavirus-positive specimens of the combined specimens from the two hospitals are shown by month in Fig. 1c. Overall, there seemed to be no appreciable difference in rotavirus-positive rates by month between the MMC and SK hospitals. The proportion of rotavirus-positive specimens in all diarrhea specimens obtained in the two hospitals by month was 10 to 20%, except in January, September, October, and December 1988 and in January 1989. An increase in the proportion of rotavirus diarrhea also seemed to correspond to the spread of the flood after August: the rotavirus-positive rates were 30, 54, 45, and 25% in September, October, and December 1988 and January 1989, respectively.

Table 1 shows the number of patients who were positive for rotavirus in their stool specimens in the two hospitals. The ages of patients with rotavirus diarrhea in the DMC hospital were mostly under 2 years. In the MMC hospital, the same age preference was observed for patients between January and July 1988. However, after August, rotavirus diarrhea in patients over 2 years of age increased greatly. In the SK hospital, in which only adult patients were treated, rotavirus diarrhea was most frequent among those patients who were in their twenties and thirties during the study period.

Antigenic characterization of rotaviruses. Subgrouping and serotyping of group A rotaviruses in stools collected from patients with diarrhea were carried out with an ELISA with subgroup- and serotype-specific monoclonal antibodies. As shown in Table 2, of a total of 141 rotavirus-positive specimens obtained from patients in the MMC hospital, 101 (71.6%) could be subgrouped. Of these, 36 (35.6%) belonged to subgroup I, 62 (61.4%) belonged to subgroup II, and 3 (3.0%) showed a dual subgroup specificity (I plus II). The subgroup specificity of the other 40 specimens were not determined. As to the serotype specificity, 51 (36.2%) of the 141 virus-positive specimens were serotyped and 90 (63.8%) remained undetermined. Among the 51 specimens whose serotypes were determined, 20 (39.2%) were assigned to serotype 2, 15 (29.4%) were assigned to serotype 1, 9 (17.6%) were assigned to serotype 4, and 1 (2.0%) were assigned to serotype 3. Six specimens (11.8%) were doubly reactive to either serotypes 2 and 4 or serotypes 1 and 2.

The results of subgrouping and serotyping of the 104 rotavirus-positive specimens obtained from adult patients in the SK hospital are shown in Table 3. The patterns of subgroup and serotype distribution of rotaviruses from adult patients were somewhat similar to those of viruses from the pediatric patients mentioned above. The subgroup specificities of 78 (75%) of 104 rotaviruses were determined. Of these, 33 (42.3%) belonged to subgroup I, 41 (52.6%) belonged to subgroup II, and 4 (5.1%) showed a dual subgroup specificity (I plus II). The subgroup specificity of the remaining 26 was left undetermined. Of the 104 virus-positive specimens, 48 (46.2%) were assigned to any one serotype or the combination of two serotypes, while 56 (53.8%) remained undetermined. Of the 48 specimens whose serotypes were determined, serotypes 1 and 2 were the most frequent ones (31.3 and 27.1%, respectively), followed by serotype 4 (25%). Eight specimens (16.7%) showed dual serotype specificity of serotype 2 plus 4, serotype 1 plus 2, or serotype 1 plus 4. No specimens of serotype 3 were detected.

Sixteen stool specimens containing strains that were subgrouped but not serotyped with the ELISA and that had a single RNA electropherotype (see RNA electropherotypes) were inoculated onto primary monkey kidney cell cultures. Five strains were successfully isolated. On the basis of the results of the ELISA and neutralization tests, one each was determined to be subgroup I-serotype 2 and subgroup II-serotype 3, two were determined to be subgroup II-serotype 4, and one (strain B221) was determined to be subgroup II-serotype 9.

The results of the antigenic characterization of rotaviruses obtained from pediatric patients in the DMC hospital in Dhaka were reported elsewhere (1).

RNA electropherotypes of rotavirus. Of the 298 stool specimens that were positive for rotavirus from the three hospi-

TABLE 2. Distribution of subgroups and serotypes of human rotavirus from diarrheic children admitted to the MMC hospital, Mymensingh, Bangladesh

Subgroup	No. of specimens	No. of specimens assigned to serotype							No. of specimens of undetermined serotype
		1	2	3	4	2+4[a]	1+2[a]	Total	
I	36	0	20	0	2	2	0	24	12
II	62	13	0	1	6	1	0	21	41
I+II	3	0	0	0	0	1	2	3	0
Undetermined	40	2	0	0	1	0	0	3	37
Total	141	15	20	1	9	4	2	51	90

[a] These specimens showed high optical density values (≥0.6) to both serotypes and could not be assigned to a single serotype according to the criteria for serotype determination.

TABLE 3. Distribution of subgroups and serotypes of human rotavirus from adult diarrheic patients admitted to the SK hospital, Mymensingh, Bangladesh

Subgroup	No. of specimens	No. of specimens assigned to serotype								No. of specimens of undetermined serotype
		1	2	3	4	2+4[a]	1+2[a]	1+4[a]	Total	
I	33	0	13	0	2	2	1	0	18	15
II	41	14	0	0	9	1	0	1	25	16
I+II	4	0	0	0	0	1	2	0	3	1
Undetermined	26	1	0	0	1	0	0	0	2	24
Total	104	15	13	0	12	4	3	1	48	56

[a] These specimens showed high optical density values (≥0.6) to both serotypes and could not be assigned to a single serotype according to the criteria for serotype determination.

tals in Mymensingh and Dhaka, 199 specimens whose quantities were large enough to allow the extraction of viral RNA were subjected to RNA electropherotype determination by PAGE. RNA bands were visible in 159 specimens (79.9%), while no bands were detectable in the other 40 specimens (20.1%). Of these 159 specimens, 82 showed clearly stained electrophoretic patterns of viral RNA, which enabled us to further classify them into distinct electropherotypes (Fig. 2). In 50 specimens, electropherotypes could not be determined, since in these specimens the staining of RNA segments, especially segments 10 and 11, was not clear enough to permit their assignment to specific electropherotypes. The remaining 27 specimens showed mixed RNA patterns (mixed RNA electropherotypes), indicating the presence of extra electrophoretic bands of viral RNA (Table 4).

Usually electrophoretic patterns of human rotavirus RNA are grouped into two major categories: i.e., a long electropherotype, in which RNA segment 11 migrates rapidly, and a short electropherotype, in which the same segment migrates slowly. In Fig. 2, the 14 distinct migration patterns of viral RNA belonging to the long electropherotype (A through N) and three distinct RNA patterns belonging to the short electropherotype (X through Z) that were found in this study are arranged by the order of detection. Differentiation among similar electropherotypes was made by coelectrophoresis of viral RNAs (data not shown). All of the virus specimens that exhibited RNA electropherotypes A, C, and J were serotype 1, whereas all of those with electropherotypes E, G, H, M, and N were serotype 4. A single specimen with electropherotype B was serotype 3. As expected, all those having short electropherotypes (X, Y, and Z) were exclusively serotype 2. In contrast, serotypes of virus specimens having electropherotypes D, F, I, K, and L remained undetermined.

Figure 3 shows several examples from virus specimens of mixed RNA electropherotypes found in this study. Although all of these are examples of mixed patterns of long and short electropherotypes, they contained 12 to 17 genomic segments of rotavirus, in contrast to the 11 RNA segments characteristic of rotavirus.

The monthly occurrence of rotavirus specimens from the three hospitals having each RNA electropherotype or a mixed electropherotype is shown in Fig. 4. In this figure, the study period is divided provisionally into two parts: the period between January and July 1988 prior to the spread of flooding in Bangladesh (the preflood period) and the period between August 1988 and January 1989 following the spread of flooding (the flood period). A rotavirus with electropherotype A was the most common in the present study. It was detected not only in the DMC hospital in Dhaka but also in the MMC and SK hospitals in Mymensingh (located about 120 km north of Dhaka). Furthermore, it was detected in the MMC and SK hospitals in Mymensingh during the entire period of the present study. Viruses having long electropherotypes B through K and short electropherotype X were detected only in the preflood period. Of these, viruses with electropherotypes C, E, and X prevailed in both cities.

The strain with electropherotype L was first detected in July before the flood and again in September after the spread of the flooding. In contrast, viruses with long electropherotypes M and N and short electropherotypes Y and Z constituted the major epidemic strains during the flood period in

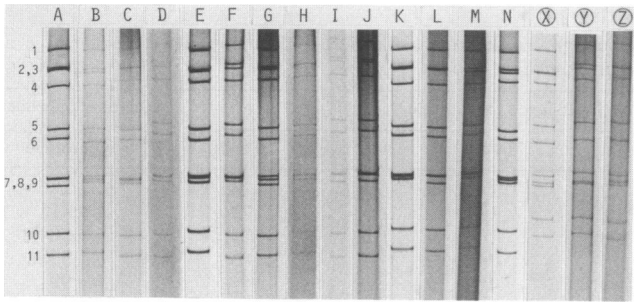

FIG. 2. RNA electropherotypes of rotaviruses obtained in Bangladesh.

TABLE 4. Electropherotyping of rotavirus RNA and the occurrence of mixed RNA electropherotype by month

Mo	No. of specimens examined	No. of specimens with RNA electropherotype		
		Identified	Unidentified	Mixed pattern (%)[a]
Jan.	12	4	7	1 (8.3)
Feb.	4	4	0	0
Mar.	27	19	4	4 (14.8)
Apr.	1	1	0	0
May	13	12	1	0
June	2	2	0	0
July	3	2	1	0
Aug.	19	3	11	5 (26.3)
Sept.	13	7	4	2 (15.4)
Oct.	43	20	9	14 (32.6)
Nov.	0	0	0	0
Dec.	18	6	12	0
Jan.	4	2	1	1 (25)
Total	159	82	50	27

[a] The percentage of mixed-pattern specimens from January 1988 through July 1988 was (5/62) 8.1%. The percentage for August 1988 through January 1989 was (22/97) 22.7%.

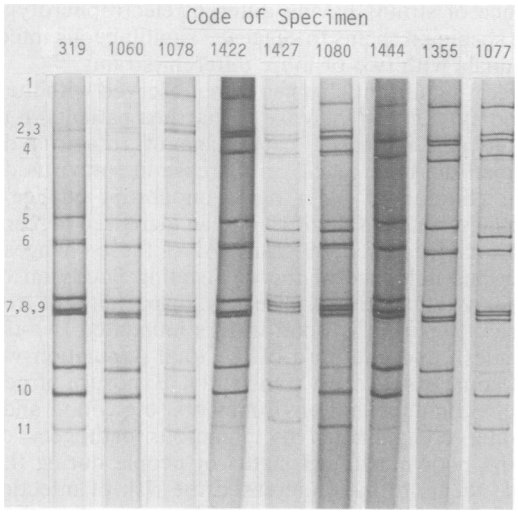

FIG. 3. Various patterns of mixed RNA electropherotypes of rotaviruses obtained in Bangladesh.

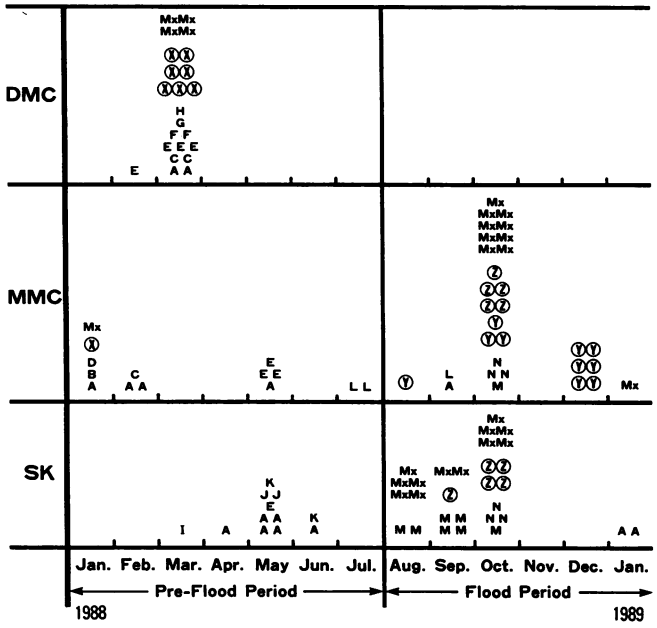

FIG. 4. Monthly change of RNA electropherotic patterns of human rotaviruses collected in three hospitals in Bangladesh. Letters enclosed within circles indicate short electropherotypes of viral RNA. Mx indicates mixed RNA electropherotypes.

Mymensingh. Thus, the results shown in this figure indicated cocirculation of diverse human rotavirus strains in Bangladesh as well as an abrupt change of epidemic strains after the devastating nationwide floods.

It was noteworthy that a number of virus specimens showed a mixed pattern of viral RNA, as seen in Fig. 4. Table 4 shows the number of specimens having a mixed RNA pattern obtained in each month. As for the specimens obtained before the flooding, 5 (8.1%) of 62 showed a mixed RNA pattern, whereas of the 97 specimens obtained after the flooding, 22 (22.7%) showed a mixed pattern. The difference in the detection rates of mixed electropherotypes between those before and after the flood was statistically significant ($P < 0.05$ by the χ^2 test).

DISCUSSION

Two outer capsid proteins, VP4 and VP7, are known to be involved in the neutralization of rotavirus. Of these, the glycoprotein VP7 (encoded by the 8th or 9th genomic segment of rotavirus) usually determines the serotype specificity (the VP7 serotype) of the virus as defined by the neutralization reaction (18, 27), whereas VP4 (encoded by the 4th RNA segment) appears to be responsible for cross-neutralization among serotypes and to play a usually minor role in serotype specificity (29). The inner capsid protein VP6 (encoded by the 6th RNA segment) is known to govern the other major antigen, i.e., subgroup, of rotavirus (14). In the present study, therefore, the serotype and subgroup specificities of rotaviruses contained in stool specimens were determined by an ELISA with serotype-specific monoclonal antibodies (directed at the VP7 of each rotavirus serotype) and subgroup-specific monoclonal antibodies (directed at the VP6 of each rotavirus subgroup).

The present study revealed that three rotavirus serotypes, 1, 2 and 4, were prevalent in the pediatric and adult populations in Mymensingh, Bangladesh. A serotype 3 strain was detected in only a single case of infantile gastroenteritis. However, as suggested by a previous report (24), the possibility remains that the serotype 3 monoclonal antibody used in this study might have missed some strains because of its limited reactivity. This limited reactivity was shown to be present by the fact that the only serotype 3 strain isolated in this study was identified by a neutralization test with specific hyperimmune serum but not by the ELISA with the monoclonal antibody. Thus, in this study, the prevalence of serotype 3 strains is considered to be underestimated. A similar survey carried out in Dhaka in the same year (1) showed that serotype 2 and 4 rotaviruses were also predominant among pediatric patients in Dhaka.

Many studies have reported the strong association between subgroup I and serotype 2 antigens and between subgroup II and serotype 1, 3, or 4 antigens of human rotavirus, although rare exceptions have been reported (19, 20). The present study substantially confirmed this association between subgroup and serotype antigens. However, in this study, several specimens whose antigenic specificity apparently does not conform with this association (Tables 1 and 2) are worthy of mention. Fourteen specimens having dual serotype specificity (six in the MMC hospital and eight in the SK hospital) were examined by PAGE. Of these specimens, 12 were found to be mixtures of virus strains having long and short RNA electropherotypes. One specimen showed an electrophoretic pattern too weak to be analyzed in detail, and one specimen (having subgroup II and serotype 1+4 antigens) showed a long electropherotype. When four specimens showing subgroup I and serotype 4 specificity (two each in the MMC and the SK hospitals) were analyzed by PAGE, they all showed mixed electropherotypes. Thus, the unusual antigenic character of selected virus specimens was thought to be mostly due to the presence of two different viruses in stool specimens. Since monoclonal antibodies specific to serotypes 8, 9, and 12 were not available, the serotyping ELISA used in this study could not detect these serotypes in stool specimens. However, in virus isolation experiments performed with selected stool specimens, one of the five isolates was found to be serotype

9, indicating the circulation of this newly described serotype in our study population.

In the present study, 146 (59.6%) of 245 specimens examined could not be serotyped. The failure of serotyping in more than half of the specimens is considered to be due to the absence of sufficient numbers of complete double-shelled virus particles in stool specimens (1), since the monoclonal antibodies used in the ELISA could react only with the individual serotype-specific neutralization epitopes on VP7 in the outer capsid layer of double-shelled particles. In this connection, the application of VP7 serotyping by using the polymerase chain reaction with serotype-specific primers is expected to greatly improve the efficiency of serotyping.

The use of serotype-specific monoclonal antibodies in an ELISA has made it possible to study more exactly the epidemiology of rotavirus serotypes. These studies, including the one presented here, have indicated the overall predominance of serotype 1 rotavirus in most of the countries in which investigations have been made (4, 10, 22, 35). At the same time, these studies suggest that differences exist in the prevalence of each serotype by locale and that yearly changes occur in the frequency of individual serotypes in the same locales (4, 22, 35).

In the present study, at least 14 different strains having long RNA electropherotypes and 3 different strains having short RNA electropherotypes were found. The strong association between the subgroup and serotype specificities of human rotavirus and the RNA electropherotype of the virus, which has been reported by many investigators (15, 17), were confirmed again in this study: strains with long RNA patterns had subgroup II and serotype 1, 3, or 4 antigens, whereas strains with short RNA patterns had subgroup I and serotype 2 antigens.

Robert Black et al. (5) described in their longitudinal studies of diarrheal diseases in Bangladesh children that the incidence of rotavirus diarrhea did not show marked seasonal variation, except for a small peak in December. In 1988, however, the record-breaking heavy rainfall brought by a monsoon seems to have seriously affected the occurrence of acute gastroenteritis. Since August, when the flooding began to spread throughout the country, the number of patients with either infantile or adult diarrhea increased greatly (Fig. 1b). It is said that government surveillance reported 1.64 million cases of diarrhea by the middle of November 1988 (16). It was noteworthy that the nationwide floods increased the percentage of rotavirus diarrhea of the diarrhea cases reported in addition to the actual number of rotavirus diarrhea cases. In October, more than half of the diarrhea cases seen in both hospitals were rotavirus-positive (Fig. 1c).

While cocirculation of diverse human rotaviruses having individual RNA electropherotypes was demonstrated in each city, several rotavirus strains (those having electropherotypes A, C, E, and X) were found in both cities studied. Furthermore, epidemic strains before the flooding contrasted strongly with those after the flooding: strains with long RNA patterns B through K and short RNA pattern X were prevalent exclusively before the flooding, while those with long patterns M and N and short patterns Y and Z formed the majority of epidemic strains during the spreading of flooding, although two strains (those with patterns A and L) prevailed during both preflood and flood periods.

It was quite unexpected that 27 (17%) of 159 virus specimens examined showed mixed RNA patterns and furthermore that the majority of them (81.5%) were detected during the flood period. A mixed viral pattern which indicates the presence of strains having different electropherotypes in a single specimen seems to suggest a simultaneous infection of individuals with two or more different strains.

Several significant changes were observed with the spread of flooding, e.g., an increase in diarrhea patients in the two hospitals, an increased percentage of rotavirus diarrhea among all diarrhea cases, an increase in rotavirus diarrhea among children of older ages, an abrupt change in the epidemic strains of rotavirus, and an increased percentage of mixed rotavirus infection cases. All of these changes reflect the serious influence of the devastating floods on the epidemiology of diarrheal diseases, especially of rotavirus diseases. Reportedly, flood waters inundated two-thirds of Bangladesh and one-third of the total population was rendered homeless, at least transiently. Exposure of people to severely contaminated environments, e.g., food and drinking water contaminated by numerous pathogens coupled with the poor nutritional status of people during the flood period, seems to have increased the risk of infection with various pathogens, including rotavirus, as well as the risk of mixed infection with two different rotaviruses.

Because of the segmented nature of the rotavirus genome, reassortment of viral genome segments is known to occur easily both in vitro and in vivo through the process of simultaneous infection with two different rotaviruses (11, 33). Conditions such as those described above, therefore, may have increased the opportunity for genetic reassortment to occur between different rotavirus strains and facilitated the appearance in nature of new reassortant viruses with unique electropherotypes.

The main clinical symptoms of patients as revealed by examination of patient records were diarrhea, vomiting, and fever. The frequency of each symptom seemed not to differ appreciably between the pediatric and adult patient groups studied. The duration and severity of each clinical symptom also did not differ significantly between the two age groups. Thus, although it has been reported that adult rotavirus infections are usually clinically mild (19), the symptoms were equally severe in both children and adults in this study, as far as hospitalized patients were concerned.

Examinations for bacterial pathogens were not carried out in the present study. However, it is most probable that diarrhea due to bacteria was also augmented during the flood period, considering the increase in overall diarrhea cases and the increased percentage of mixed infection cases with rotavirus following the spread of flooding. The general impression of clinicians that the diarrhea of patients admitted to the hospitals during the flood period was often clinically severe might also support the notion that some of the patients were infected with not only viral but also bacterial or protozoal agents.

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